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Isabel Mangas Palma

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MESTRADO INTEGRADO EM MEDICINA

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# ADRENAL INCIDENTALOMA: CLINICAL, ANALYTICAL AND IMAGING FOLLOW-UP ON A SINGLE CENTRE.

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# ABSTRACT

OBJECTIVE: The increasing incidence of adrenal incidentalomas is attributed to the widespread use of imaging techniques. Although in most cases adrenal incidentalomas are benign and non-functioning, their clinical relevance is related to the possibility of malignancy or hormonal production. Their follow-up approach is controversial since their natural history is unknown. Our aim is to characterize patients with adrenal incidentalomas from a single tertiary-care centre in Portugal (Centro Hospitalar do Porto).

DESIGN AND METHODS: This observational, retrospective and longitudinal study included all patients with adrenal incidentaloma observed at an outpatient clinic specialized in adrenal gland disease between January 2014 and March 2018. Adrenal incidentaloma was defined as an adrenal mass detected on imaging not performed for suspected adrenal disease. Parameters regarding demographic features and imaging, clinical and analytical evaluations were collected.

RESULTS: A total of 142 patients were included with a mean age at diagnosis of 59.4 years; 54.9% were women. Most subjects (61.3%) had non-functioning adrenal incidentalomas; 29.2% possible autonomous cortisol secretion, 5.7% autonomous cortisol secretion, 2.8% primary hyperaldosteronism and 0.9% pheochromocytoma. A non-contrast attenuation coefficient >10 Hounsfield units was found in at least one mass in 8.7% patients and a maximum diameter ≥40 mm in 6.4%. Median follow-up duration was 2.0 years. An increase in the number of masses was observed in 21.1% patients and a decrease in 7.8%; an increase in maximum diameter >5 mm was observed in 23.3% patients and a decrease in 4.4%. Neither primary nor metastatic adrenal cancer were found. Moreover, 54.4% patients developed comorbidities; median time from diagnosis to their development was 4.0 years. Additionally, five cardiovascular events were registered; median time from diagnosis to these events was 2.0 years. Regarding cortisol production, during follow-up, 11.6% patients showed a worsening and 23.3% an improvement. No patients developed primary hyperaldosteronism, pheochromocytoma or overt endocrine disease.

CONCLUSIONS: Our results suggest that in most cases adrenal incidentalomas remain stable throughout imaging follow-up and that malignant transformation is rare. The risk of developing comorbidities appears to be high, however prevention may improve these patients' outcomes since few cardiovascular events were observed. Concerning hormonal follow-up, cortisol production may vary over time, contrarily to other adrenal hormones.

KEYWORDS: Adrenal incidentaloma; Obesity; Diabetes Mellitus; Dyslipidaemias; Hypertension; Atherosclerosis.

# RESUMO

**OBJETIVOS:** O aumento da incidência de incidentalomas da suprarrenal é atribuído ao uso crescente das técnicas de imagem como meios complementares de diagnóstico. Apesar de, na maioria dos casos, estes incidentalomas serem benignos e não funcionantes, a sua relevância clínica deve-se principalmente à possibilidade de malignidade ou produção hormonal. O protocolo de seguimento é controverso já que a sua história natural não está esclarecida. O objetivo deste trabalho é caracterizar os doentes com incidentaloma da suprarrenal de um hospital terciário terciários em Portugal (Centro Hospitalar do Porto).

**DESENHO E METODOLOGIA:** Estudo observacional, retrospetivo e longitudinal que incluiu todos os doentes com incidentaloma da suprarrenal observados na consulta externa específica para doença da suprarrenal entre Janeiro de 2014 e Março de 2018. Foi definido como incidentaloma da suprarrenal uma massa detetada por estudos imagiológicos não realizados por suspeita de doença da suprarrenal. Foram registados parâmetros relativos a características demográficas e às avaliações imagiológica, clínica e analítica.

**RESULTADOS:** Foram incluídos 142 doentes com uma idade média ao diagnóstico de 59,4 anos; 54,9% eram mulheres. A maioria dos doentes (61,3%) apresentava incidentaloma não-funcionante, 29,2% possível secreção autónoma de cortisol, 5,7% secreção autónoma de cortisol, 2,8% hiperaldosteronismo primário e 0,9% feocromocitoma. Verificou-se uma densidade >10 unidades de Hounsfield em pelo menos uma massa em 8,7% dos doentes e um diâmetro máximo ≥40 mm em 6,4%. A duração mediana do seguimento foi de 2,0 anos. Observou-se um aumento do número de massas em 21,1% dos doentes e uma diminuição em 7,8%; um aumento do diâmetro máximo >5 mm em 23,3% dos doentes e uma diminuição em 4,4%. Nenhum doente apresentava ao diagnóstico ou desenvolveu durante o seguimento cancro da suprarrenal primário ou metastático. Adicionalmente, 54,4% dos doentes desenvolveram comorbilidades; o tempo mediano entre o diagnóstico e o seu aparecimento foi de 4,0 anos. Registaram-se cinco eventos cardiovasculares e o tempo mediano entre o diagnóstico e estes eventos foi de 2,0 anos. Relativamente à produção de cortisol, durante o seguimento, 11,6% dos doentes revelaram um agravamento e 23,3% uma melhoria. Nenhum doente desenvolveu hiperaldosteronismo primário, feocromocitoma ou doença endócrina clínica.

**CONCLUSÕES:** Os nossos resultados sugerem que a maioria dos doentes com incidentaloma da suprarrenal permanece imagiologicamente estável durante o seguimento e que a transformação maligna é rara. O risco de desenvolvimento de comorbilidades parece ser elevado, mas a prevenção poderá melhorar os resultados, já que a incidência de eventos cardiovasculares foi baixa. A produção de cortisol poderá variar ao longo do tempo, o que não se verifica com as restantes hormonas produzidas pela suprarrenal.

PALAVRAS-CHAVE: Incidentaloma da suprarrenal; Obesidade; Diabetes Mellitus; Dislipidemia; Hipertensão; Aterosclerose.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACS | autonomous cortisol secretion |
| AI | adrenal incidentaloma |
| BMI | body mass index |
| c-HDL | high-density lipoprotein cholesterol |
| c-LDL | low-density lipoprotein cholesterol |
| CT | computed tomography scan |
| DM | diabetes mellitus |
| HU | Hounsfield unit |
| IQR | interquartile range |
| LNSC | late night salivary cortisol |
| MR | magnetic resonance scan |
| MSC | morning salivary cortisol |
| OCS | overt Cushing's syndrome |
| PACS | possible autonomous cortisol secretion |
| PHA | primary hyperaldosteronism |
| P | probability value |
| SD | standard deviation |
| UFC | 24-hour urinary free cortisol |
| US | ultrasonography |
| 1 mg DST | overnight low-dose dexamethasone suppression test |

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# INTRODUCTION

Adrenal incidentalomas (AIs) are adrenal masses detected in imaging studies not performed for suspected adrenal disease.[1] In recent years, AIs have become progressively relevant in clinical practice as their incidence and prevalence considerably increased. This is believed to be the result of both population-ageing as well as technological advances which resulted in a widespread use of imaging techniques.[2-6] Based on imaging and autopsy studies, it is estimated that the prevalence of AIs is 1-10%, increasing with age, with a peak of incidence in the fifth to seventh decades of life.[5, 6] Although its prevalence is superior in women in imaging series, in autopsy ones no sex difference was found.[5, 6]

AIs form a heterogeneous group that comprises a wide range of entities, simultaneously including functioning and non-functioning (i.e. hormone producing or not), benign and malignant, and adrenal and extra-adrenal aetiologies.[1] Although the majority of cases are non-functioning benign incidentalomas (most often adenomas), the clinical relevance of AIs is related to the possibility of malignancy or hormone production (most often cortisol).[1, 6, 7]

Subclinical Cushing's syndrome or, according to the latest European Society of Endocrinology guideline, autonomous cortisol secretion (ACS) is defined by the presence of biochemical evidence of an increased cortisol production without the classical clinical features of hypercortisolism that define overt Cushing's syndrome (OCS).[1, 8] Analogously to OCS, ACS has been associated with an increased risk of osteoporosis and fragility fractures, and several metabolic abnormalities such as insulin resistance, type 2 diabetes mellitus (DM), obesity and hypertension, all of which are associated with an increased risk of cardiovascular events and mortality.[1, 9-21] However, ACS is much more prevalent than OCS. It is estimated that ACS is present in 1.0-29.0% of AIs and, thereby, its prevalence in adult population is between 0.2-2.0%.[1, 22, 23] The high prevalence of ACS combined with the associated risk of comorbidities and mortality justify its relevance. Interestingly, recent studies have also linked non-functioning AIs with a higher risk of comorbidities and cardiovascular events, raising the hypothesis that these two categories are different phases of the same process and not distinct entities.[24-26]

The European Society of Endocrinology guideline published in 2016 on the management of AIs has received some criticism regarding the recommendations on follow-up of patients with non-functioning benign lesions and with possible autonomous cortisol secretion (PACS) without comorbidities.[27, 28] These recommendations are markedly different from those of the previous guideline by the American Association of Clinical Endocrinologists and the American Association of Endocrine Surgeons of 2009, however the quality of the evidence supporting them is classified as very low.[1, 27, 28] In addition, European Society of Endocrinology guideline itself proposes two studies about biochemical and radiological follow-up which further confirms how insufficient data about these themes there is and validates this criticism. The first one is a long-term study with annual biochemical work-up of patients with AI to clarify if such a long-term hormonal assessment is justified and determine the true incidence of relevant diseases. The second is a large, long-term study to define whether or not ACS is associated with increased mortality and other hard clinical endpoints.[1] In fact, more evidence is needed to define the ideal follow-up strategy that must weigh the risk of complications related to radiation exposure from repeated computed tomography scans (CTs), the psychological impact and the economic costs against the benefits to patients.[29]

The aims of our study are (1) to evaluate imaging, clinical and analytical (biochemical and hormonal) features at diagnosis and throughout follow-up in patients with AI from a single tertiary-care centre in Portugal and (2) compare patients with non-functioning AI with those with PACS or ACS regarding these features.

# SUBJECTS AND METHODS

## STUDY DESIGN AND SUBJECTS

This observational longitudinal study was carried out at Hospital de Santo António – Centro Hospitalar do Porto (HSA–CHP) – a tertiary-care and university centre. The records regarding all consecutive patients observed at an outpatient clinic specialized in adrenal gland disease between January 2014 and March 2018 were retrospectively analysed. All adult patients with AI were included. Patients whose diagnosis was made by imaging studies done for (1) symptoms or signs related to adrenal hormone excess or deficiency, (2) investigation of hypertension, (3) screening in the context of hereditary syndromes causing adrenal tumours, (4) evaluation of extra-adrenal malignancies (diagnosis, staging or follow-up) or (5) an otherwise suspected adrenal disease were excluded. Patients who did not meet the criteria of having a CT and a clinical, a biochemical and a hormonal evaluation were also excluded.

Parameters regarding demographic features and imaging, clinical, biochemical and hormonal evaluations were collected. The first office visit's date and the specialty of the physician who referred the patient were also registered. All data was obtained by perusal of clinical records (paper and/or computer) as well as CT and laboratory reports.

The periodicity and parameters included in these patients' evaluation was guided by different protocols based on the current guidelines at the time. Regarding imaging evaluation, this protocol defines that all AIs diagnosed by ultrasonography (US) or magnetic resonance scan (MR) are evaluated by CT up to 6 months after the first office visit. Moreover, patients diagnosed by CT but without a report or with an incomplete report and that we do not have access to the diagnostic exam for re-evaluation by the study's radiologist (R.M.) also repeat CT up to 6 months after the first office visit. Follow-up protocol included a CT performed 1, 3 and 5 years after the first office visit until 2017, when the European Society of Endocrinology guidelines were adopted.[1] Since then, the study's radiologist analyses all first CTs and those patients whose CT is unequivocally benign do not repeat it unless any clinical or analytical abnormality that requires further imaging is identified throughout follow-up. Regarding clinical and analytical evaluation, our institution's protocol includes two initial evaluations during the first 6 months after the first office visit, followed by an annual evaluation unless any abnormality that requires further investigation is identified in the initial screening or throughout follow-up.

## IMAGING EVALUATION

The AI diagnosis' date, the diagnostic imaging modality and the reason for performing this exam were recorded. Registered imaging characteristics included the number of masses; their localization (right or left adrenal gland or bilateral); their diameter, defined as its maximum transverse diameter; and their density, defined as non or pre-contrast attenuation coefficient and categorized as ≤ or >10 Hounsfield units (HU). When non or pre-contrast attenuation coefficient was >10 HU, absolute and relative washouts after contrast were also recorded.

All these imaging characteristics were obtained from CTs which were not always performed by the same equipment nor evaluated by the same radiologist. In case of doubt regarding the report or incomplete reports, the images were re-evaluated by the study's radiologist.

## CLINICAL EVALUATION

According to our protocol, in each office visit, symptoms and signs of adrenal hormone excess or deficiency, prescribed medication, smoking habits, cardiovascular events, height (only on the first visit), body weight, waist circumference and systolic and diastolic blood pressure are registered.

Demographic data included age and sex. Clinical data included all the registered parameters previously referred as well as deceases and their causes. Former smoker was defined as having ceased smoking at least 6 months before the assessment. Cardiovascular events encompassed myocardial infarction, transient ischemic attack, stroke and acute ischemia in the context of peripheral artery disease.

## ANALYTICAL EVALUATION

Following our protocol, the first evaluation is done within 3 months after the first office visit. Blood samples are obtained between 8-9 h a.m., after an 8-h overnight fasting. Saliva samples are collected between 11-12 h p.m. (late night) and 7-8 h a.m. (morning). 24-h urine samples are collected according to standard recommendations. All patients receive detailed instructions on how to properly perform saliva sampling and urine collection at home. This first evaluation includes general biochemical parameters as creatinine, urea, sodium, potassium, chloride, alanine aminotransferase, aspartate aminotransferase, fasting glucose, haemoglobin $A_{1c}$ – only in patients with DM – total cholesterol, low-density lipoprotein cholesterol (c-LDL), high-density lipoprotein cholesterol (c-HDL) and triglycerides. It also includes a hormonal evaluation with (1) morning and late night salivary cortisol (MSC and LNSC, respectively) and

24-h urinary free cortisol (UFC) to evaluate cortisol secretion; (2) plasma metanephrines and 24-h urinary fractionated catecholamines and metanephrines to exclude pheochromocytoma; and (3) plasma aldosterone and direct renin concentrations to exclude primary hyperaldosteronism (PHA). Since 2017, these two last parameters are only evaluated in patients with hypertension or unexplained hypokalaemia[1].

The second evaluation occurs within 3 months after the second office visit. It includes an overnight low-dose dexamethasone suppression test (1 mg DST): 1 mg of dexamethasone (Decadron®) is administrated between 11-12 h p.m. on the day before and blood samples for cortisol measurement are obtained in the following morning between 8-9 h a.m.

In the ensuing annual evaluations, blood, saliva and urine samples are collected following the same protocol. These evaluations included the general biochemical and hormonal parameters previously referred. Since 2017, only general biochemical parameters are evaluated, unless any hormonal or clinical abnormality that needs further investigation is identified[1].

All biochemical and hormonal studies were performed at HSA–CHP laboratory. Creatinine, urea, glucose, total cholesterol, c-HDL and triglycerides were measured by standard procedures, with intra and inter-assay coefficients of variation <2.8% and <3.9%, respectively. Sodium, potassium and chloride were measured by indirect potentiometry, with intra and inter-assay coefficients of variation <0.7% and <1.5%, respectively. All these parameters were measured using an automated autoanalyzer (Cobas 8000, Roche Diagnostics, Mannheim, Germany). Friedewald formula was used to calculate c-LDL.

Saliva samples were collected using cotton swabs from Salivette® tubes (Sarstedt, Nümbrecht, Germany). Salivary and serum cortisol were measured using an automated electrochemiluminescence immunoassay (Cobas e601, Roche Diagnostics, Mannheim, Germany). Cross-reactivity with dexamethasone was negligible and intra and inter-assay coefficients of variation were 9.3 and 14.2%, respectively. UFC was measured using a chemiluminescent microparticle immunoassay (ARCHITECT i2000SR, Abbott), with intra and inter-assay coefficients of variation of 5.3 and 6.2%, respectively. Plasma metanephrines were measured with a chromatography-tandem mass spectrometry assay, with intra and inter-assay coefficients of variation of 6.6 and 8.3%, respectively. Urinary fractionated catecholamines and metanephrines were measured with a high-performance liquid chromatography method with electrochemical detection, with intra and inter-assay coefficients of variation of 2.9 and 4.1% for catecholamines and 2.7 and 4.4% for metanephrines, respectively. Aldosterone and renin were measured with a radioimmunoassay (CISBIO, Codolet, France), with intra and inter-assay coefficients of variation of 8.3 and 8.4% for aldosterone and 3.6 and 5.0% for renin, respectively.

## DIAGNOSTIC CRITERIA

An **AI** was defined as an adrenal mass detected on imaging not performed for suspected adrenal disease.[1]

OCS, ACS, PACS, pheochromocytoma and PHA were diagnosed based on the European Society of Endocrinology Clinical Practice Guidelines and our institution's protocols.

**OCS** diagnostic criteria were (1) presence of clinical features of hypercortisolism and (2) at least two of the following: LNSC >0.35 µg/dL in two measurements, UFC above the upper limit of the normal range for the assay (176 µg/24h) in two measurements and serum cortisol >1.8 µg/dL after 1 mg DST.[8] **ACS** was defined as serum cortisol >5.0 µg/dL after 1 mg DST and lack of clinical features of hypercortisolism and **PACS** was defined as serum cortisol >1.8 and ≤5.0 µg/dL after 1 mg DST and lack of clinical features of hypercortisolism.[1]

**Pheochromocytoma** diagnostic criteria were (1) plasma metanephrines or 24-h urinary fractionated catecholamines or metanephrines ≥3 folds the upper limit of the normal range for the assay and/or (2) diagnostic histopathologic characteristics after surgical resection.[30]

Regarding **PHA**, plasma aldosterone (pg/mL) and direct renin (pg/mL) concentrations measured at the same time were used to calculate plasma aldosterone/renin ratio and a ratio >57 was considered a positive screening. This ratio was a screening test and if positive it was repeated after minimizing confounding factors (for instance, medications). Saline infusion test was performed to confirm diagnosis.[31]

**Hypertension** was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90mmHg on at least two blood pressure measurements per visit and on at least two visits[32] and/or prescription of any antihypertensive medication.

**Dyslipidaemia** was defined as c-LDL ≥115 mg/dL and/or c-HDL ≤40 mg/dL in men or ≤45 mg/dL in women and/or triglycerides ≥150 mg/dL[33] and/or prescription of any antidyslipidaemic medication.

**DM** was defined as plasma glucose ≥126mg/dL in at least two measurements[34] and/or prescription of any antidiabetic medication. **Prediabetes** was defined as a plasma glucose ≥100 and <126 mg/dL.[34] **Insulin resistance** was estimated using (1) the homeostasis model assessment, calculated according to the equation 1;[35] and using (2) the triglyceride/glucose index, calculated according to the equation 2.[36]

$$\frac{\text{insulin } (\mu\text{IU/ml}) \times \text{plasma glucose (mg/dL)}}{405} \qquad \text{(Equation 1)}$$

$$\ln\left(\frac{\text{triglycerides (mg/dL) x plasma glucose (mg/dL)}}{2}\right) \qquad \text{(Equation 2)}$$

**Obesity** was defined as a body mass index (BMI) ≥30 kg/m$^2$ and being **overweight** as BMI ≥25 and <30 kg/m$^2$.[7] **Waist circumference** was considered abnormal if ≥94 cm in men and ≥80 cm in women.[33]

## SURGERY CRITERIA

A diameter ≥40 mm, functioning AIs (PHA, pheochromocytoma and OCS) or features suggesting malignancy on CT (irregular margins, non- or pre-contrast attenuation coefficient >10 HU, absolute washout ≤60% and relative washout ≤40% both after contrast, among others[1]) were indications for surgical treatment.

## STATISTICAL ANALYSIS

Regarding analysis of imaging features, when a patient had more than one mass, diameter was defined by the mass with the greatest diameter and density was considered ≤10 HU if all masses had a density ≤10 HU and >10 HU if one of the masses had a density >10 HU. Follow-up analysis only included patients with a follow-up duration ≥1 year.

Statistical analysis was performed using IBM SPSS Statistics, version 25.0. Regarding descriptive statistics, continuous variables were reported as mean (standard deviation (SD)) or median (interquartile range (IQR)), depending on whether distribution was considered normal or not, respectively, by Shapiro-Wilk test. For categorical variables, descriptive statistics were reported as absolute frequency (relative frequency, %). Continuous variables were analysed using parametric (t-test or one-way ANOVA) or non-parametric (Mann-Whitney U, Kruskal-Wallis H and Wilcoxon tests) tests when appropriate. Categorical variables were analysed using Chi-square test or Fisher's exact test when appropriate. Statistical significance was defined as a probability value (P) <0.05.

## ETHICS STATEMENT

This study was approved by the ethical committee of CHP in the 21st of February 2018 and was conducted in agreement with the principles of the Declaration of Helsinki. Informed consent was obtained for all patients.

# RESULTS

From January 2014 to April 2018, 245 patients were observed at our outpatient clinic. After analysing their records, we identified 173 patients with AI. Within these, only 142 had a CT and a clinical, a biochemical and a hormonal evaluation and, thereby, were enrolled in our study (Figure 1).

Among patients enrolled, 54.9% were women. Mean age at diagnosis was 59.4 years; and 75.4% patients were between the fifth to seventh decades of life (Table I).

In total, 90 patients had a follow-up duration ≥1 year, with a median duration of 2.0 (IQR 1.0-5.0) years. Throughout follow-up, 24 patients were lost: 1 died in the context of decompensated heart failure; 2 underwent unilateral adrenalectomy; and 21 discontinued follow-up (6 decided by the physician and 15 on their own account).

## IMAGING EVALUATION

In 79.6% patients, AIs were diagnosed by CT or MR (111 by CT and 2 by MR); in the remaining cases AIs were diagnosed by US. The most frequent reason for diagnostic imaging was gastrointestinal symptoms or disease (50.0% patients).

The majority of patients (83.1%) had unilateral masses, 53.4% of which on the left adrenal gland. Left sided masses were more likely to be identified by CT or MR than by US (51.3% vs. 17.2%; $P=0.004$) (Table II). No differences were found between patients with left, right or bilateral masses in regard to sex, age and BMI.

Most patients (79.6%) had a single adrenal mass; 14.8% had two adrenal masses. No significant differences were found between patients with different numbers of masses in regard to age, sex, BMI and diagnostic method. The number of masses identified by CTs performed during follow-up was compared with the number of masses at first CT: 19 (21.1%) patients had an increase; and 7 (7.8%) a decrease, in 3 of which the only mass disappeared. Median time from diagnosis to the increase or decrease in the number of masses was 2.3 (IQR 1.4-3.9) and 4.6 (IQR 2.4-5.1) years, respectively. No differences were found between patients whose number of masses increased, decreased or did not change concerning age, sex, BMI, diameter and density at diagnosis.

Mean diameter was 21.9 mm. Diameter was significantly higher in bilateral masses than in left ones (27.5 (SD 14.4) vs. 20.4 (SD 12.7) mm; $P=0.026$) and in patients with two or more masses than in those with only one (26.3 (SD 13.3) vs. 20.7 (SD 11.4) mm; $P=0.012$). It was also significantly higher in AIs diagnosed by US than in those diagnosed by CT or MR (25.7 vs. 20.9

mm; P=0.019). Concerning diameter, no differences were found in regard to sex and no correlations were found concerning age or BMI. In 2.8% subjects, diameter was <10 mm, all diagnosed by CT; in 6.4%, diameter was ≥40 mm.

All diameters measured in CTs performed during follow-up were compared with the diameter measured in first CT: 21 (23.3%) patients had an increase >5 mm, seven of which >10 mm; and 4 (4.4%) had a decrease >5 mm, one of which >10 mm. Median time from diagnosis to the detection of this increase or decrease was 2.4 (IQR 1.6-4.7) and 1.5 (IQR 1.2-2.9) years, respectively. No association was found between a change in diameter >5 mm and a change in number of masses. There were also no differences between patients whose diameter increased >5 mm, decreased >5 mm or did not change >5 mm concerning age, sex, BMI, diameter and density at diagnosis. Moreover, an increase of diameter to a value ≥40 mm was observed in 3 (3.3%) patients; none of them had a density >10 HU and their mean diameter at diagnosis was 33 (7.2) mm.

In 18.4% patients no density was reported by the radiologist and we did not have access to the images. Among the remaining patients, 8.7% had a density >10 HU. One of these patients did not perform a contrast enhanced CT since he had a suspicion of pheochromocytoma. The remaining nine had an absolute washout >60% and a relative washout >40%. No significant differences were established between patients whose density at diagnosis was >10 HU and those whose density was ≤10 HU in regard to age, sex or diameter.

An imaging worsening (defined as a change in density from ≤10 HU to >10 HU) was detected in 8 (8.9%) patients. Median time from diagnosis to worsening was 2.0 (IQR 1.3-3.9) years. All these patients revealed an absolute washout >60% and a relative washout >40% in contrast enhanced CTs. No relation was found between worsening and a change in diameter >5 mm or number of masses. There were also no differences between patients with or without an imaging worsening concerning age, sex and diameter at diagnosis.

Finally, neither primary nor metastatic adrenal cancer was found in our study at diagnosis or during follow-up.

## CLINICAL AND BIOCHEMICAL EVALUATIONS

Median time from diagnosis to the first visit at our outpatient clinic was 5.0 (IQR 3.0-10.0) months; this time was >12 months in 33 (23.2%) patients. In total, 43.7% patients were referred by family physicians (Figure 2).

At diagnosis, 69.6% patients had a BMI ≥25 kg/m$^2$ (41.3% of which had obesity); 66.2% dyslipidaemia; 56.3% hypertension; 34.5% tobacco smoking habits; 33.1% glucose intolerance

(70.2% of which had DM); and 9.9% history of cardiovascular events (Table III). Only 59 patients had a waist circumference measurement, the majority (n=56, 94.9%) presented a waist circumference ≥94 or 80, depending on sex. Within these, 14 (25.0%) had a BMI <25 kg/m$^2$, 23 (41.1%) were overweight and 19 (33.9%) obese.

Throughout follow-up, 54.4% patients developed comorbidities, 22.4% of which developed two comorbidities. Prediabetes (28 new cases), hypertension (16) and dyslipidaemia (14) were the most frequent (Figure 3). Median time from diagnosis to the development of these comorbidities was 5.0 (IQR 2.0-10.0) years. Among patients who developed comorbidities, 39 (79.6%) already had comorbidities at diagnosis. Patients who did not have comorbidities at diagnosis had a significantly higher proportion of comorbidities' development (83.3% vs. 50.0%; P=0.031). BMI was significantly lower in patients who developed comorbidities (26.7 (SD 4.4) vs. 29.3 (SD 4.9) kg/m$^2$; p=0.039). No differences were found between patients who developed comorbidities throughout follow-up and those who did not regarding age, sex, first 1 mg DST, change in cortisol production, diameter at diagnosis and change in number of masses or in diameter >5mm. In total, five cardiovascular events were registered and median time from diagnosis to these events was 2.0 (IQR 1.5-5.0) years.

Clinical and biochemical features at diagnosis and 5 years after the diagnosis are shown in Table IV. When patients with available data from both periods were selected, we found an increase of 6.3 mg/dL in fasting glucose (n=34; P=0.029) and a decrease of 5.5 mg/dL in c-HDL (n=17; P=0.044). No significant differences were found regarding the other features.

## HORMONAL EVALUATION

Overall, 106 patients performed a complete initial hormonal evaluation (i.e. the two evaluations within 6 months after the first office visit). Most patients (61.3%) had non-functioning AIs, 29.2% PACS, 5.7% ACS, 2.8% PHA and 0.9% pheochromocytoma (Figure 4). Two patients with PHA and the one with pheochromocytoma simultaneously presented an abnormal cortisol production: the former had PACS and ACS and the latter had PACS.

Hypertension was present at diagnosis in all patients with PHA and absent in the one with pheochromocytoma. Further, no patients with PHA revealed hypokalaemia (potassium <3.5 mmol/L) at diagnosis.

During follow-up, only 43 patients performed another complete hormonal evaluation. Regarding cortisol production, a worsening was observed in 11.6% patients, nevertheless no patients developed OCS. Conversely, 23.3% patients showed an improvement. Moreover, 7.0% patients presented an oscillating profile and the remaining 58.1% persisted stable. No differences were found between patients whose cortisol production worsened, improved, oscillated and did not change throughout follow-up regarding age, sex, BMI, first 1 mg DST, diameter at diagnosis and change in the number of masses or in diameter >5 mm. Regarding the production of other hormones, no changes were registered: no patients developed pheochromocytoma or PHA. These changes resulted in a final distribution of 48.8% non-functioning AIs, 37.2% PACS, 9.3% ACS and 4.7% PHA.

Hormonal parameters at diagnosis and 5 years after the diagnosis are shown in Table IV. When patients with available data from both periods were selected, no significant differences were found between diagnosis and 5 years after diagnosis regarding these parameters.

## SURGERY

A total of 16 (11.3%) patients had indication for surgery, 13 at diagnosis and three during follow-up: nine had a diameter ≥40 mm; three PHA; and one pheochromocytoma and a density >10 HU (Figure 1).

Only three patients underwent surgery (unilateral adrenalectomy): the one with pheochromocytoma at diagnosis and the others during follow-up (both with a diameter ≥40 mm, one with PACS and the other with a non-functioning AI). The pheochromocytoma diagnosis was confirmed by histopathology; the others histopathology was cortical hyperplasia and adenoma, respectively. None of these patients experienced corticotroph insufficiency after surgery.

The remaining ten patients who did not undergo surgery either are waiting for surgery, had bilateral masses, refused surgery, were lost to follow-up (transferred to other institutions) or reduced mass diameter to <40 mm.

## NON-FUNCTIONING *VERSUS* POSSIBLE OR AUTONOMOUS CORTISOL SECRETION

Regarding demographic features, patients with PACS or ACS were older than those with non-functioning either at diagnosis or 5 years after (63.4 vs. 57.2 years; P=0.006 and 68.3 years vs. 60.2 years; P=0.006, respectively) (Table V). No differences between patients with PACS or ACS and those with non-functioning were found in regard to sex, comorbidities and clinical and biochemical features either at diagnosis or 5 years after. Moreover, no differences were found regarding imaging features – location, number of masses, diameter and density at diagnosis (Table VI) and change in number of masses or in diameter >5 mm throughout follow-up.

Among the 90 patients included for follow-up analysis, 77 patients performed a complete initial hormonal evaluation (Figure 3). Within these, excluding the 2 patients with PHA, 64.0% were non-functioning and the remaining PACS or ACS. Throughout follow-up, comparing non-functioning vs. PACS or ACS, 60.4% vs. 48.1% patients developed comorbidities (P=0.304). Within these patients, 20.7% vs. 38.5% developed two comorbidities (P=0.270). Prediabetes (15 vs. 9 new cases), hypertension (10 vs. 4) and dyslipidaemia (9 vs. 4) were the most frequent. Median time from diagnosis to development of these comorbidities was 5.5 (IQR 1.0-10.0) vs. 4.0 (IQR 2.0-10.0) years (P=0.868). Among patients who developed comorbidities, 11 (84.6%) vs. 22 (75.9%) had at least one comorbidity at diagnosis (P=0.695). Moreover, 4 (11.8%) patients with PACS or ACS had a cardiovascular event vs. 1 (1.9%) patients with non-functioning. Median time from diagnosis to these events was 2.0 (IQR 1.3-2.8) years for patients with PACS or ACS. The only event in patients with non-functioning occurred 7.0 years after diagnosis.

Since the role of MSC, LNSC, UFC and inversion of the circadian rhythm (evaluated with MSC and LNSC) in the diagnostic approach of AI is not as well established as it is for OCS, we performed a subanalysis to compare patients with PACS or ACS with those with non-functioning AI regarding these variables and evaluate their agreement with 1 mg DST. We verified that although LNSC at diagnosis was significantly lower in patients with non-functioning vs. those with PACS or ACS (0.118 vs. 0.446; P=0.005), there were no significant differences concerning MSC and UFC. When analysing the agreement between 1 mg DST and LNSC at diagnosis, we obtained a Kappa coefficient of 0.3 when using 1.8 μg/dL as cut-off for 1 mg DST and of 0.2 when using 5.0 μg/dL. It was not possible to analyse the agreement between 1 mg DST and UFC at diagnosis since there were no values of UFC above the upper limit of normal range (176 μg/24h).

Among the 93 patients who had at least 1 evaluation of MSC and LNSC at diagnosis, 14 patients had inversion of the circadian rhythm on the first evaluation. No significant differences were established between patients with PACS or ACS and those with non-functioning AIs regarding inversion of the circadian rhythm (8 (25.0%) patients vs. 6 (9.8%); P=0.069).

# DISCUSSION

In our series, there were slightly more women (54.9%) than men. Regarding sex distribution evidence is conflicting: some studies are in agreement with ours and described a tendency to a higher prevalence in women (55.2-73.2%);[6, 19, 37-46] others in men (52.7-61.0%);[47-49] and others, including autopsy series, reported no differences.[6, 50-53] This higher prevalence in women is generally attributed to a referral bias since it is believed that abdominal diagnostic procedures are performed more frequently in women.[6]

Mean age at diagnosis (59.4 years) was consistent with the literature (52-69 years).[6, 19, 37-45, 47-49, 52-55] Additionally, the majority of patients were in the fifth to seventh decades of life, that corresponds to AI's incidence peak.[6] This distribution may be either a consequence of a higher number of diagnostic procedures in older patients (requested by routine or disease) or a result of development of adrenal masses with age.[38] The latter is in agreement with autopsy series[38, 50, 51] and its mechanism may involve ischemia followed by compensatory hyperplasia.[38, 52, 56, 57]

## IMAGING EVALUATION

The majority of AIs were diagnosed by CT, followed by US and MR. This distribution is in agreement with some studies, conducted in 3 different countries (Croatia, Japan and Sweden) and published between 2002 and 2016;[40, 42, 44, 49] but not with a widely cited study conducted in Italy and published in 2000, which refers US as the most frequent diagnostic technique.[38] Since the majority of studies after 2000 excluded cases diagnosed by US and/or MR,[41, 47, 48, 58] evidence is unclear. Nevertheless, the difference between these studies could be explained by a more widespread use of CT and MR nowadays and by geographic differences that may be associated with different accessibilities to imaging modalities.

Globally, reasons for diagnostic imaging in our study were similar to those described in literature.[38, 40, 42, 47, 49, 52, 55, 59] Gastrointestinal symptoms or disease was the most frequent cause followed by genitourinary symptoms or disease. This is supported by several studies that described general check-up (when this category was considered) and gastrointestinal or genitourinary symptoms (particularly, abdominal pain and haematuria) as the most frequent reasons for abdominal imaging in the setting of AI's diagnosis.[38, 40, 42, 47, 49, 52, 55, 59]

In our series, the majority of patients (83.1%) had unilateral masses, which is consistent recent studies (75.2-92.5%).[19, 38-42, 45-47, 49, 52, 53, 55, 58-60] Unilateral masses were slightly more frequent on the left gland (53.4%) than on the right. Evidence regarding this side distribution is conflicting: some recent studies are in agreement with ours and described unilateral masses' tendency to be more frequently located on the left gland (60.5-67.3%);[39, 41, 47, 49, 58] others (mostly older) on the right (55.9-72.9%);[6, 19, 38, 46, 61, 62] and others, including CT and autopsy series, reported no differences.[6, 37, 54]

Moreover, left sided masses were more likely to be identified by CT or MR than by US. Our results are in agreement with others that showed US detected right-sided masses more often than left-sided ones[38, 49] and that CT detected them at similar rates.[38] This difference is due to the fact that US is able to visualize the right adrenal gland better than the left, being less efficient than CT and MR in detecting masses on the left side.[6, 16, 38, 58, 63, 64] This fact could also elucidate why in older studies masses were more frequently located on the right adrenal gland, since US was generally the most frequent diagnostic method.[47]

Additionally, diameter was significantly higher in bilateral masses than in left ones; this is in agreement with Li *et al.* who found that bilateral masses were larger but not with Vassilatou *et al.* who found no differences.[52, 60] No differences were found between patients with right, left and bilateral masses concerning sex, age and BMI. Regarding age and BMI, this is in agreement with previous studies; considering sex, Li *et al.* found that bilateral masses were more frequent in men, but the other studies found no differences.[40, 52, 59, 60]

In our study, most patients (79.6%) had a single mass, in a proportion similar to that described by Cho *et al.* (88.7%).[47] As far as we know, there are no studies that analysed the change in the number of masses throughout follow-up. In our study, the proportion of patients that presented this change was low (28.9%) and an increase in the number of masses was more frequent than a decrease (21.1 vs. 7.8%). Interestingly, in 3.3% patients the only mass disappeared. No parameter that distinguished patients concerning change in number of masses was identified.

Mean diameter obtained in our study (21.9 mm) is similar to that described by some recent studies (17.2-25.0 mm).[19, 39, 40, 42, 45, 47-49, 52, 53, 58] However, it is lower than that described in older studies (30-35 mm).[6] This may be due to the multiple AI definitions (that may or may not include masses <10 mm, for instance), a different distribution of diagnostic modalities (as discussed previously, it appears that CT is replacing US as the most frequent modality, increasing the sensibility of the diagnosis) and to an improved resolution of imaging methods, particularly CT an MR.[39] Furthermore, no differences were found in mean diameter in regard to sex, age or BMI, which is in agreement with Kastelan *et al.*[40]

Additionally, the proportion of cases with a diameter <10 mm (2.8%) and ≥40 mm (6.4%) is in agreement with other studies (1.7-6.2% and 7.0-8.6%, respectively).[19, 39, 41, 47, 49] The fact that all masses with a diameter <10 mm were detected by CT is also in agreement with Tabuchi *et al.* who observed that the majority of masses measuring <10 mm were diagnosed by

CT.[49] Moreover, but contrarily to Tabuchi *et al.*, diameter was significantly higher in masses identified by US.[49] These last two findings reflect the greater resolution and sensibility of CT.

Results concerning change in diameter during follow-up in our study were similar to those from other studies with a follow-up duration between 1.6-3.0 years: 72.3% of patients in our study vs. 78.2% in other studies presented no change in diameter; 23.3% vs. 2.7-16.3% increased (7.8% vs. 0-0.6% >10 mm); and 4.4% vs. 1.7-6.9% decreased.[19, 40, 42, 47] In our study the proportion of patients with an increase in diameter was slightly higher. This may be due to the fact that CTs were neither analysed by the same radiologist (which is associated with a certain inter-observer variability) nor always performed in the same equipment. Similarly to our study, in other studies, age, sex, BMI, diameter and density at diagnosis were not parameters that distinguished patients concerning change in diameter.[19, 47] Moreover, we found an increase to ≥40 mm in a higher proportion of patients (2.1% vs. 0%).[40] These differences may be also due to the methodological aspects already mentioned.

The proportion of patients with no density reported by the radiologist (18.4%) was higher than that observed in other studies (2.8-7.0%).[40, 53] Therefore, the importance of increasing the referring physicians as well as radiologists awareness about the significance of a complete and detailed report or images access during the patient's management becomes evident, as to assure there is no unnecessary radiation exposure. The proportion of patients with a density >10 HU (8.7%) was lower than that described by other studies (24.0%-56.6%).[40, 41] The absence of a significant difference between these patients and those with a density ≤10 HU in regard to diameter is not supported by Kastelan *et al.* that described that the first group of patients had a larger diameter.[40]

In our knowledge, there are no studies that analysed the change in density throughout follow-up. As with number of masses and diameter, our study revealed a low proportion of patients with worsening of these imaging features throughout follow-up.

Finally, the fact that neither primary nor metastatic adrenal cancer were found at diagnosis in our study is compatible with one study,[49] but not with others, that describe malignancy in 0.4-29.0% of patients.[6, 38-42, 47, 48, 52, 54] This variability between studies may be due to differences in sample size and inclusion criteria, namely including or not patients with history of extra-adrenal cancer. In our study patients whose diagnosis was made by imaging studies performed during evaluation of extra-adrenal malignancies were excluded. Furthermore, the absence of malignant transformation is compatible with recent studies.[19, 29, 40, 45, 48]

## DISCUSSION

### CLINICAL AND BIOCHEMICAL EVALUATION

Almost half of our patients (43.7%) were referred to our clinic by a primary care physician. These results call attention to the necessity of educating these physicians regarding the importance of promptly referring these patients to an endocrinologist. Time from diagnosis to the first visit to our outpatient clinic was >12 months in 23.2% patients, which once more illustrates the importance of bringing to attention an early referral. In our knowledge, there are no studies that evaluated time between diagnosis and first Endocrinology clinical evaluation nor the medical specialties of the physicians who referred the patients.

The proportion of patients with different comorbidities at diagnosis in our study is in agreement with those described in literature: 69.6% of patients in our study vs. 26.5-54.0% in other studies had an abnormal BMI (23.2% vs. 26.5-28.6% obesity); 66.2% vs. 14.2-48.9% dyslipidaemia; 56.3% vs. 38.3-69.3% hypertension; 33.1% vs. 15.0-37.3% glucose intolerance (23.2% vs. 13.3-19.9% DM); and 9.9% vs. 8.7-26.5% history of cardiovascular events.[19, 40, 42, 45, 47, 49, 52] Although our study reveals a tendency to slightly higher proportions of these comorbidities, there is great variability between studies that could be attributed to differences regarding inclusion criteria, referral pattern and even diagnostic criteria.

Few patients had a waist circumference measurement at diagnosis, thus our results should be interpreted with caution. Nevertheless, it is important to highlight that 25% of patients had an abnormal waist circumference, despite having a normal BMI. This, allied to the fact that waist circumference is an important cardiovascular risk modifier, emphasizes the importance of measuring it in all patients.

During follow-up, 54.4% patients developed comorbidities but only five cardiovascular events occurred. Moreover, regarding clinical and biochemical characteristics, no differences were found when comparing data at diagnosis and 5 years after the diagnosis except for plasma glucose and c-HDL which worsened. Although this worsening was statistically significant, it was not clinically significant and could be explained by the ageing of the sample.

This high prevalence and incidence of comorbidities should be interpreted keeping in mind the mean age of our sample and that age is an important risk factor for all these comorbidities or events. Since we did not have a control group, they could not be attributed to AI and advanced age by itself could explain the high prevalence and incidence of comorbidities registered. Additionally, all these patients were being submitted to primary, secondary and even tertiary prevention measures which may interfere in the natural history of these diseases by improving its course or even delaying its beginning. This may explain the low incidence of cardiovascular events.

## HORMONAL EVALUATION

In our study, the majority of patients had non-functioning AIs (61.3%), which is compatible with what is described in the literature (68.0-88.6% of patients).[1, 6, 19, 38-41, 47-49, 52, 54] PACS or ACS was the most common functioning AI in our sample, which, along with the distribution of functioning AIs, is also consistent with what is described in the literature: 34.9% of patients in our study vs. 1.0-29.0% in other studies had PACS or ACS; 2.8% vs. 0.4-11.5% had PHA; and 0.9% vs. 0.9-14.0% had pheochromocytoma.[1, 6, 19, 38-41, 47-49, 52, 54] The prevalence of PACS or ACS is highly variable between studies and this can be attributed to the different diagnostic tests and criteria used. In fact, there is no gold standard for the diagnosis and authors may use only one test or a combination of tests (generally, 1 mg DST is used as screening test and if positive may be complemented by other tests). Additionally, the cut-off values vary, for instance 1.8 and 5.0 μg/dL in 1 mg DST are used. Coexistence of PACS or ACS with PHA has also been observed in Tabuchi *et al.* and in a similar proportion (1.4 vs. 1.3%).[49]

Within patients with PHA, the proportion of patients with hypokalaemia at diagnosis in our study was lower than that described Hong *et al.* (0 vs. 37.1%).[48]

The change in cortisol production in our study is compatible with recent studies with a follow-up duration between 1.9-7.5 years: a worsening was observed in 11.6% of patients in our study vs. 0-12.9% in other studies and an improvement in 23.3% vs. 0-28.0%.[19, 40, 45, 47, 48, 65] In our knowledge, no study reported an oscillating pattern of cortisol production. Once more, this variability between studies may be attributed to different diagnostic criteria. Moreover, the non-existence of dexamethasone dosing restricts the interpretation of 1 mg DST results.

Notwithstanding the limitations of 1 mg DST, cortisol production appeared to variate during follow-up, which supports the contemporary view that cortisol production in AI may be increasing or intermittent over time.[24, 66]

No parameter that distinguished patients concerning change in cortisol production was identified. This is contrary to Morrelli *et al.* who found that worsening was associated to larger mass diameter at diagnosis.[65]

Additionally, the absence of development of OCS, pheochromocytoma or PHA is compatible with recent studies,[19, 40, 45, 48] except for Cho *et al.* that described de development of 1 (0.7%) case of pheochromocytoma.[47]

## SURGERY

The proportion of patients submitted to surgery (2.1%) was lower than those described by other studies (6.1-47.7%).[19, 20, 39-42, 47, 49, 52] This difference may be attributed to the stricter surgery criteria used in our institution. Among patients with indication for surgery, the proportion of each indication and the reasons for not undergoing surgery were similar to other studies.[41, 47] Slight differences concerning the proportion of each indication may be attributed mainly to the absence of malignancy in our study and the fact that PACS or ACS were not considered an indication for surgery.

## NON-FUNCTIONING VERSUS POSSIBLE OR AUTONOMOUS CORTISOL SECRETION

The literature concerning differences between patients with PACS or ACS and those with non-functioning AI is rather contradictory. Regarding age, some studies are in agreement with ours and described a greater age in patients with PACS or ACS;[45, 65] contrarily, Tabuchi *et al.* described a younger age in these patients;[49] and other studies reported no age differences.[19, 40, 47] Concerning sex, most recent studies are in agreement with ours and found no significant differences;[19, 40, 45, 49, 65] only Cho *et al.* described a higher proportion of women in patients with PACS or ACS.[47] Regarding imaging characteristics, some studies are in agreement with ours and found no differences concerning mass location,[45, 47] while others described a higher proportion of bilateral masses in patients with PACS or ACS.[6, 40, 52, 59, 60, 65] To our knowledge, only Cho *et al.* described differences regarding number of masses and, as in our study, no differences were found.[47] Moreover, some studies are in agreement with ours and found no differences concerning diameter,[19, 47] and others described a higher diameter in patients with PACS or ACS.[6, 40, 45, 49, 65] Additionally, and contrarily to our study which found no differences, some studies described a higher density in patients with PACS or ACS.[40, 47] It is important to note that some of these studies are slightly different from ours as they included in the same group other functioning AIs besides PACS or ACS, although their proportions were very low.[47, 49]

Furthermore, as already mentioned, several studies reported that cortisol excess in PACS or ACS is associated with an increased cardiovascular risk by inducing multiple metabolic derangements,[9, 11, 12, 65, 67] that include overweight and obesity, prediabetes and DM,[1, 45, 65] hypertension[1, 49] and dyslipidaemia.[1, 19] On the contrary, our study found no differences between patients with PACS or ACS and those with non-functioning AI at diagnosis and 5 years after regarding the prevalence of comorbidities or cardiovascular events, which is partially or totally supported by some recent studies, that found only one difference or no differences between these groups of patients regarding these comorbidities.[19, 40, 45, 47, 49] Additionally, in our study, no significant differences were found between patients with PACS or ACS and those with non-functioning AI concerning the development of comorbidities. Moreover, no differences were found regarding presence of comorbidities at diagnosis in patients who later developed comorbidities during follow-up and concerning time from diagnosis to development of these comorbidities. Nevertheless, patients with PACS or ACS appeared to have more cardiovascular events than those from the non-functioning group.

There are at least three factors that may explain these contradictory results seen in literature. Firstly, it is important to keep in mind the heterogeneity among diagnostic criteria previously discussed. Secondly, it was hypothesized that non-functioning, PACS and ACS AI have a common origin and only represent distinct phases of the same phenomena, in other words, a continuum of cortisol production that may result in an increasing cardiovascular risk. In fact, several studies have found that non-functioning AI also have an increased risk of comorbidities (particularly insulin resistance and DM) and cardiovascular events and that this risk could be explained by secretion of low cortisol levels.[24-26] This new perspective not only led to the questioning of the previously defined cortisol cut-offs that were used in studies but also to the realization of how heterogeneous study groups can be regarding cortisol production. For instance, a study might have a non-functioning group with a mean cortisol production significantly lower than other study with consequent impact on its outcomes. On the other hand, this increased cardiovascular risk of non-functioning AI may also bring back to discussion an older theory about the origin of AI that proposed ischemia (mainly in the context of atherosclerosis) as a triggering factor. This theory may also explain the increased risk in PACS and ACS group. Lastly, the possibility of intermittent production can influence studies' outcomes, since this phenomenon may not be detected.

Concerning the subanalysis to evaluate the performance of other diagnostic tests in comparison with 1 mg DST, we verified that although LNSC at diagnosis was significantly higher in PACS or ACS group, there were no significant differences concerning MSC and UFC. When analysing the agreement between 1 mg DST and LNSC at diagnosis, we obtained low Kappa coefficients using 1.8 μg/dL or 5.0 μg/dL as cut-offs. Moreover, it was not possible to analyse the agreement between 1 mg DST and UFC at diagnosis since there were no values of UFC above the upper limit of normal range. This is in agreement with Kastelan *et al.* that described that only 25% of the patients with ACS (defined as serum cortisol levels >3 μg/dL in 1 mg DST) had elevated UFC and with Di Dalmazi *et al.* that found no significant differences regarding UFC between patients with non-functioning AI, PACS and ACS.[40, 45] These results concerning UFC may be explained by the low sensitivity of this method and by errors in the 24h-urine sampling. Those regarding LNSC may also be explained by sampling errors. In fact, although all sampling methods are explained in detail to the patients, they are complex and time-consuming.

Among the 93 patients who had at least 1 evaluation of MSC and LNSC at diagnosis, 14 patients had inversion of the circadian rhythm on the first evaluation. Patients with PACS or ACS revealed a tendency to a higher proportion of inversion of the circadian rhythm (P=0.069), which is in agreement Debono *et al.*[68] In fact, these results could also be influenced by sampling errors already discussed, particularly the exchange in the MSC and LNSC tubes that can easily occur.

## LIMITATIONS

Our study presents some limitations: (1) the retrospective design; (2) the fact that it only included patients from a single centre (a tertiary care and university institution) which may constitute a selection bias; (3) the unavailability of some data; (4) the short median follow-up duration; (5) the size of the sample; (6) the fact that CTs were neither analysed by the same radiologists nor performed in the same equipment.

# CONCLUSIONS

In conclusion, our results suggest that most AIs remain stable during imaging follow-up and that malignancy is a rare event. This is supported by other studies and justifies the current European guidelines recommendation of no further imaging evaluation after an unequivocally benign initial CT.

Many studies have associated a higher risk of metabolic comorbidities and cardiovascular events not only in PACS or ACS but also in non-functioning AI, which suggests that globally patients with AI have a higher cardiovascular risk. This high risk is compatible with our results that also suggest that prevention may improve these patients' outcomes. Nevertheless, more studies are needed to evaluate the importance of a clinical and biochemical follow-up oriented towards cardiovascular risk in patients with AI and in which context this follow-up should occur (primary, secondary or tertiary-care centres) so that current recommendations can be adjusted.

According to our results and most recent studies, development of pheochromocytoma and PHA is a rare event. This supports the recommendation of current European guidelines to only evaluate RAA and urinary catecholamines and metanephrines at first visit and repeat merely if these initial results are abnormal. Regarding cortisol production, our study supports the contemporary view that this production may be increasing or intermittent over time. In this context, a single evaluation of cortisol production at diagnosis seems insufficient so that it is important to design studies in the future that can evaluate the relevance of monitoring cortisol secretion and which periodicity has the best cost-effectiveness ratio. Moreover, it is imperative to evaluate the role of UFC, LNSC and inversion of the circadian rhythm (with MSC and LNSC) in AIs' study, since our results suggest they have a limited role. Additionally, the utility of dexamethasone dosing in the interpretation of 1 mg DST should be analysed.

We hope that our study will function as a starting point for not only a larger Portuguese multicentre study which would allow a more accurate characterization of the epidemiology of AIs in Portugal but also a larger prospective study regarding AI's follow-up and its associated long-life cardiovascular risk.

# DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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# TABLES

Table I: Demographic and imaging features at diagnosis.

| | n | % |
|---|---|---|
| **Demographic features** | | |
| Women | 78 | 54.9 |
| **Age, years** | | |
| Mean (SD) | 59.4 | (12.1) |
| <40 | 5 | 3.5 |
| 40-69 | 107 | 75.4 |
| >69 | 30 | 21.1 |
| **Imaging features** | | |
| **Imaging modality that led to diagnosis** | | |
| Computed tomography scan | 111 | 78.2 |
| Ultrasonography | 29 | 20.4 |
| Magnetic resonance scan | 2 | 1.4 |
| **Indication for performing the diagnostic imaging study** | | |
| Gastrointestinal symptoms or disease | 71 | 50.0 |
| Genitourinary symptoms or disease | 22 | 15.5 |
| Cardiopulmonary symptoms or disease | 16 | 11.3 |
| Others* | 17 | 12.0 |
| Unknown | 16 | 11.3 |
| **Mass location** | | |
| Unilateral | 118 | 83.1 |
| Left adrenal gland | 63 | 53.4 |
| Right adrenal gland | 55 | 46.6 |
| Bilateral | 24 | 16.9 |
| **Number of masses** | | |
| One | 113 | 79.6 |
| Two | 21 | 14.8 |
| Three | 7 | 4.9 |
| Four | 1 | 0.7 |
| **Maximum diameter, mm**** | | |
| Mean (SD) | 21.9 | (12.0) |
| < 10 | 4 | 2.8 |
| 10-19 | 63 | 44.7 |
| 20-29 | 49 | 34.8 |
| 30-39 | 16 | 11.3 |
| ≥ 40 | 9 | 6.4 |
| **Non-contrast attenuation coefficient, Hounsfield units**** | | |
| Described | 115 | 81.6 |
| ≤ 10 | 105 | 91.3 |
| > 10 | 10 | 8.7 |
| Not described | 26 | 18.4 |

N=142. SD: standard deviation; *Other indications included trauma, anaemia, syncope, weight loss and abdominal aorta aneurysm; **Data concerning maximum diameter and non-contrast attenuation coefficient were not available for 1 patient because we did not have access to the full report or the images.

Table II: First computed tomography scan features by diagnostic imaging modality.

| | CT or MR (n=113) | | US* (n=29) | | P-value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| **Mass location** | | | | | 0.004 |
| Left adrenal gland | 58 | 51.3 | 5 | 17.2 | |
| Right adrenal gland | 39 | 34.5 | 16 | 55.2 | |
| Bilateral | 16 | 14.2 | 8 | 27.6 | |
| **Number of masses** | | | | | 0.415 |
| One | 92 | 81.4 | 21 | 72.4 | |
| Two | 14 | 12.4 | 7 | 24.1 | |
| Three | 6 | 5.3 | 1 | 3.4 | |
| Four | 1 | 0.9 | 0 | 0 | |
| **Maximum diameter, mm** | | | | | 0.115 |
| Mean (SD) | 20.9 | (12.0) | 25.7 | (11.5) | 0.019 |
| < 10 | 4 | 3.5 | 0 | 0 | |
| 10-19 | 55 | 48.7 | 8 | 28.6 | |
| 20-29 | 37 | 32.7 | 12 | 42.9 | |
| 30-39 | 12 | 10.6 | 4 | 14.3 | |
| ≥ 40 | 5 | 4.4 | 4 | 14.3 | |

CT: computed tomography scan; MR: magnetic resonance scan; SD: standard deviation; US: ultrasonography; *Data concerning maximum diameter was not available for 1 patient because we did not have access to the full report or the images.

Table III: Demographic features and comorbidities at diagnosis and 5 years after diagnosis.

| | At diagnosis (n=142) | | 5 years after (n=62) | |
|---|---|---|---|---|
| | n | % | n | % |
| **Comorbidities** | | | | |
| **Altered body mass index*** | 80 | 69.6 | 36 | 58.1 |
| Overweight | 47 | 58.8 | 20 | 55.6 |
| Obesity | 33 | 41.3 | 16 | 44.4 |
| **Dyslipidaemia** | 94 | 66.2 | 48 | 77.4 |
| Evaluated | 69 | 73.4 | 26 | 54.2 |
| Controlled | 12 | 17.4 | 8 | 30.8 |
| Hypercholesterolaemia | 26 | 37.7 | 12 | 46.2 |
| Hypertriglyceridemia | 9 | 13.0 | 2 | 7.7 |
| Hypocholesterolaemia HDL | 7 | 10.1 | 2 | 7.7 |
| Combined | 15 | 21.7 | 2 | 7.7 |
| Not evaluated | 25 | 26.6 | 22 | 45.8 |
| **Hypertension** | 80 | 56.3 | 42 | 67.7 |
| Evaluated | 64 | 80.0 | 31 | 73.8 |
| Controlled | 29 | 45.3 | 11 | 35.5 |
| SBP 140-159 and/or DBP 90-99 | 26 | 40.6 | 15 | 48.4 |
| SBP 160-179 and/or DBP 100-109 | 8 | 12.5 | 5 | 16.1 |
| SBP ≥ 180 and/or DBP ≥ 110 | 1 | 1.6 | 0 | 0 |
| Not evaluated | 16 | 20.0 | 11 | 26.2 |
| **Tobacco smoking habits** | 49 | 34.5 | 17 | 27.4 |
| Former smoker | 10 | 20.4 | 2 | 11.8 |
| Current smoker | 39 | 79.6 | 15 | 88.2 |
| **Glucose intolerance** | 47 | 33.1 | 26 | 41.9 |
| Prediabetes | 14 | 29.8 | 14 | 53.8 |
| Diabetes | 33 | 70.2 | 12 | 46.2 |
| **History of cardiovascular events** | 14 | 9.9 | 4 | 6.5 |
| Coronary heart disease | 7 | 50.0 | 3 | 75.0 |
| Cerebrovascular disease | 2 | 14.3 | 1 | 25.0 |
| Peripheral artery disease | 2 | 14.3 | 0 | 0 |
| Multiple territories | 3 | 21.4 | 0 | 0 |

DBP: diastolic blood pressure; HDL: high-density lipoprotein; SBP: systolic blood pressure; *27 missing at diagnosis and 15 missing 5 years after diagnosis.

Table IV: Clinical and biochemical features at diagnosis and 5 years after diagnosis.

| | At diagnosis (n=142) | | | 5 years after (n=62) | | |
|---|---|---|---|---|---|---|
| | n | Mean | SD | n | Mean | SD |
| **Clinical evaluation** | | | | | | |
| Body mass index (kg/m²) | 115 | 28.0 | 4.9 | 47 | 28.1 | 4.2 |
| Waist circumference (cm) | 59 | 101.7 | 12.0 | 2 | 104.0 | 4.2 |
| Systolic blood pressure (mmHg) | 114 | 134.9 | 17.6 | 47 | 136.2 | 15.8 |
| Diastolic blood pressure (mmHg) | 114 | 78.6 | 10.7 | 47 | 77.4 | 10.2 |
| **General biochemical evaluation** | | | | | | |
| Fasting glucose (mg/dL) | 115 | 104.5 | 27.4 | 41 | 98.9 | 29.6 |
| Total cholesterol (mg/dL) | 92 | 189.0 | 44.2 | 29 | 193.6 | 30.2 |
| LDL cholesterol (mg/dL) | 83 | 114.1 | 39.0 | 27 | 115.5 | 28.3 |
| HDL cholesterol (mg/dL) | 87 | 52.6 | 14.3 | 27 | 58.7 | 15.6 |
| Triglycerides (mg/dL) | 93 | 119.5 | 77.0 | 29 | 107.7 | 42.9 |
| Homeostasis model assessment index* | 32 | 3.3 | (2.6-6.9) | 5 | 3.1 | (1.9-4.9) |
| Triglyceride/glucose index | 93 | 8.5 | 0.6 | 29 | 8.5 | 0.5 |
| **Hormonal evaluation** | | | | | | |
| 1 mg DST (µg/dL) | 50 | 1.9 | 1.6 | 17 | 2.5 | 1.4 |
| 24-h urinary free cortisol (µg/24h) | 85 | 47.2 | 33.7 | 22 | 44.7 | 32.3 |
| Morning salivary cortisol (µg/dL) | 57 | 0.515 | 0.319 | 7 | 0.530 | 0.287 |
| Late night salivary cortisol (µg/dL) | 54 | 0.214 | 0.280 | 8 | 0.208 | 0.145 |
| Plasma aldosterone/renin ratio | 80 | 24.5 | 44.5 | 11 | 36.7 | 41.3 |
| Plasma metanephrine (pmol/L) | 2 | 420.1 | 325.4 | 8 | 198.0 | 58.7 |
| Plasma normetanephrine (pmol/L) | 2 | 1081.3 | 264.0 | 8 | 447.5 | 206.5 |
| 24-h urinary adrenaline (nmol/24h) | 86 | 27.9 | 28.5 | 22 | 35.4 | 33.7 |
| 24-h urinary noradrenaline (nmol/24h) | 92 | 244.8 | 128.8 | 23 | 274.1 | 163.1 |
| 24-h urinary dopamine (nmol/24h) | 92 | 1245.0 | 659.5 | 23 | 1162.6 | 618.7 |
| 24-h urinary metanephrine (nmol/24h) | 90 | 470.7 | 271.0 | 23 | 451.7 | 344.4 |
| 24-h urinary normetanephrine (nmol/24h) | 90 | 1506.6 | 646.1 | 23 | 1665.0 | 947.7 |

1 mg DST: overnight low-dose dexamethasone suppression test; HDL: high-density lipoprotein; LDL: low-density lipoprotein; SD: standard deviation; *Median, Interquartile range.

Table V: Demographic, clinical and analytical features at diagnosis and 5 years after diagnosis grouped by cortisol production.

| | | Non-functioning AI* | | | PACS or ACS** | | | P-value |
|---|---|---|---|---|---|---|---|---|
| **Demographic features** | | N | n | % | N | n | % | |
| Women | At diagnosis | 65 | 38 | 58.5 | 37 | 20 | 54.1 | 0.666 |
| | 5 years after | 34 | 23 | 67.6 | 18 | 10 | 55.6 | 0.389 |
| Mean age (SD), years | At diagnosis | 65 | 57.2 | (11.9) | 37 | 63.4 | (13.0) | **0.006** |
| | 5 years after | 34 | 60.2 | (11.0) | 18 | 68.3 | (13.0) | **0.006** |
| **Comorbidities** | | N | n | % | N | n | % | |
| Dyslipidaemia | At diagnosis | 65 | 46 | 70.8 | 37 | 26 | 70.3 | 0.958 |
| | 5 years after | 34 | 29 | 85.3 | 18 | 16 | 88.9 | 1.000 |
| Glucose intolerance | At diagnosis | 65 | 18 | 27.7 | 37 | 16 | 43.2 | 0.156 |
| | 5 years after | 34 | 14 | 41.2 | 18 | 9 | 50.0 | 0.542 |
| Prediabetes | At diagnosis | | 8 | 44.4 | | 3 | 18.8 | |
| | 5 years after | | 8 | 57.1 | | 5 | 55.6 | |
| Diabetes | At diagnosis | | 10 | 55.6 | | 13 | 81.3 | |
| | 5 years after | | 6 | 42.9 | | 4 | 44.4 | |
| Hypertension | At diagnosis | 65 | 32 | 49.2 | 37 | 24 | 64.9 | 0.127 |
| | 5 years after | 34 | 20 | 58.5 | 18 | 14 | 77.8 | 0.172 |
| Altered body mass index | At diagnosis | 38 | 37 | 56.9 | 37 | 21 | 56.8 | 0.987 |
| | 5 years after | 32 | 25 | 73.5 | 18 | 10 | 55.6 | 0.189 |
| Overweight | At diagnosis | | 23 | 62.2 | | 12 | 57.1 | |
| | 5 years after | | 14 | 56.0 | | 6 | 60.0 | |
| Obesity | At diagnosis | | 14 | 37.8 | | 9 | 42.9 | |
| | 5 years after | | 11 | 44.0 | | 4 | 40.0 | |
| Cardiovascular events | At diagnosis | 65 | 3 | 4.6 | 37 | 3 | 11.3 | 0.665 |
| | 5 years after | 34 | 1 | 5.6 | 18 | 2 | 11.1 | 0.272 |
| **Clinical evaluation** | | N | Mean | SD | N | Mean | SD | |
| Body mass index, kg/m² | At diagnosis | 54 | 27.9 | 4.5 | 29 | 27.8 | 5.0 | 0.849 |
| | 5 years after | 33 | 28.1 | 3.8 | 12 | 28.3 | 4.6 | 0.904 |
| Waist circumference, cm | At diagnosis | 23 | 99.6 | 11.6 | 12 | 102.7 | 8.8 | 0.430 |
| | 5 years after | 2 | 104.0 | 4.2 | 0 | | | |
| Systolic blood pressure, mmHg | At diagnosis | 54 | 134.7 | 18.3 | 29 | 135.9 | 17.7 | 0.454 |
| | 5 years after | 33 | 136.5 | 20.8 | 12 | 135.0 | 20.8 | 0.690 |
| Diastolic blood pressure, mmHg | At diagnosis | 54 | 78.6 | 11.8 | 29 | 76.3 | 8.9 | 0.362 |
| | 5 years after | 33 | 77.5 | 11.2 | 12 | 75.9 | 7.3 | 0.961 |
| **General biochemical evaluation** | | N | Mean | SD | N | Mean | SD | |
| Fasting glucose, mg/dL | At diagnosis | 55 | 101.1 | 26.0 | 29 | 108.2 | 26.1 | 0.081 |
| | 5 years after | 28 | 94.1 | 24.5 | 11 | 112.7 | 40.1 | 0.297 |
| Total cholesterol, mg/dL | At diagnosis | 47 | 195.4 | 42.7 | 22 | 180.1 | 38.7 | 0.986 |
| | 5 years after | 19 | 199.8 | 29.4 | 9 | 183.8 | 30.7 | 0.212 |
| LDL cholesterol, mg/dL | At diagnosis | 42 | 118.2 | 37.6 | 20 | 107.8 | 27.9 | 0.745 |
| | 5 years after | 18 | 117.8 | 30.0 | 9 | 110.8 | 25.4 | 0.482 |
| HDL cholesterol, mg/dL | At diagnosis | 44 | 53.0 | 14.0 | 22 | 52.0 | 10.4 | 0.353 |
| | 5 years after | 18 | 61.6 | 16.2 | 9 | 53.0 | 13.3 | 0.089 |
| Triglycerides, mg/dL | At diagnosis | 47 | 117.7 | 75.1 | 22 | 129.8 | 101.1 | 0.912 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 years after | 19 | 108.6 | 49.6 | 9 | 105.9 | 30.0 | 0.565 |
| Homeostasis model assessment index*** | At diagnosis | 17 | 4.2 | (2.8-7.8) | 9 | 4.3 | (2.7-5.7) | 0.641 |
| | 5 years after | 5 | 3.1 | (1.8-4.9) | 0 | | | |
| Triglyceride/glucose index | At diagnosis | 47 | 8.5 | 0.6 | 22 | 8.6 | 0.6 | 0.465 |
| | 5 years after | 19 | 8.4 | 0.6 | 9 | 8.5 | 0.4 | 0.415 |
| **Cortisol evaluation** | | **N** | **Mean** | **SD** | **N** | **Mean** | **SD** | |
| 1 mg DST, µg/dL | At diagnosis | 28 | 1.1 | 0.4 | 15 | 3.2 | 1.8 | **<0.001** |
| | 5 years after | 10 | 1.6 | 0.4 | 7 | 3.8 | 1.3 | **0.001** |
| 24-h urinary free cortisol, µg/24h | At diagnosis | 47 | 47.9 | 39.4 | 21 | 43.8 | 27.9 | 0.545 |
| | 5 years after | 17 | 43.0 | 25.2 | 4 | 51.2 | 61.9 | 0.869 |
| Morning salivary cortisol, µg/dL | At diagnosis | 30 | 0.499 | 0.192 | 13 | 0.611 | 0.366 | 0.139 |
| | 5 years after | 5 | 0.516 | 0.349 | 2 | 0.566 | 0.054 | 0.699 |
| Late night salivary cortisol, µg/dL | At diagnosis | 29 | 0.118 | 0.103 | 12 | 0.446 | 0.431 | **0.005** |
| | 5 years after | 5 | 0.227 | 0.165 | 3 | 0.176 | 0.130 | 0.764 |

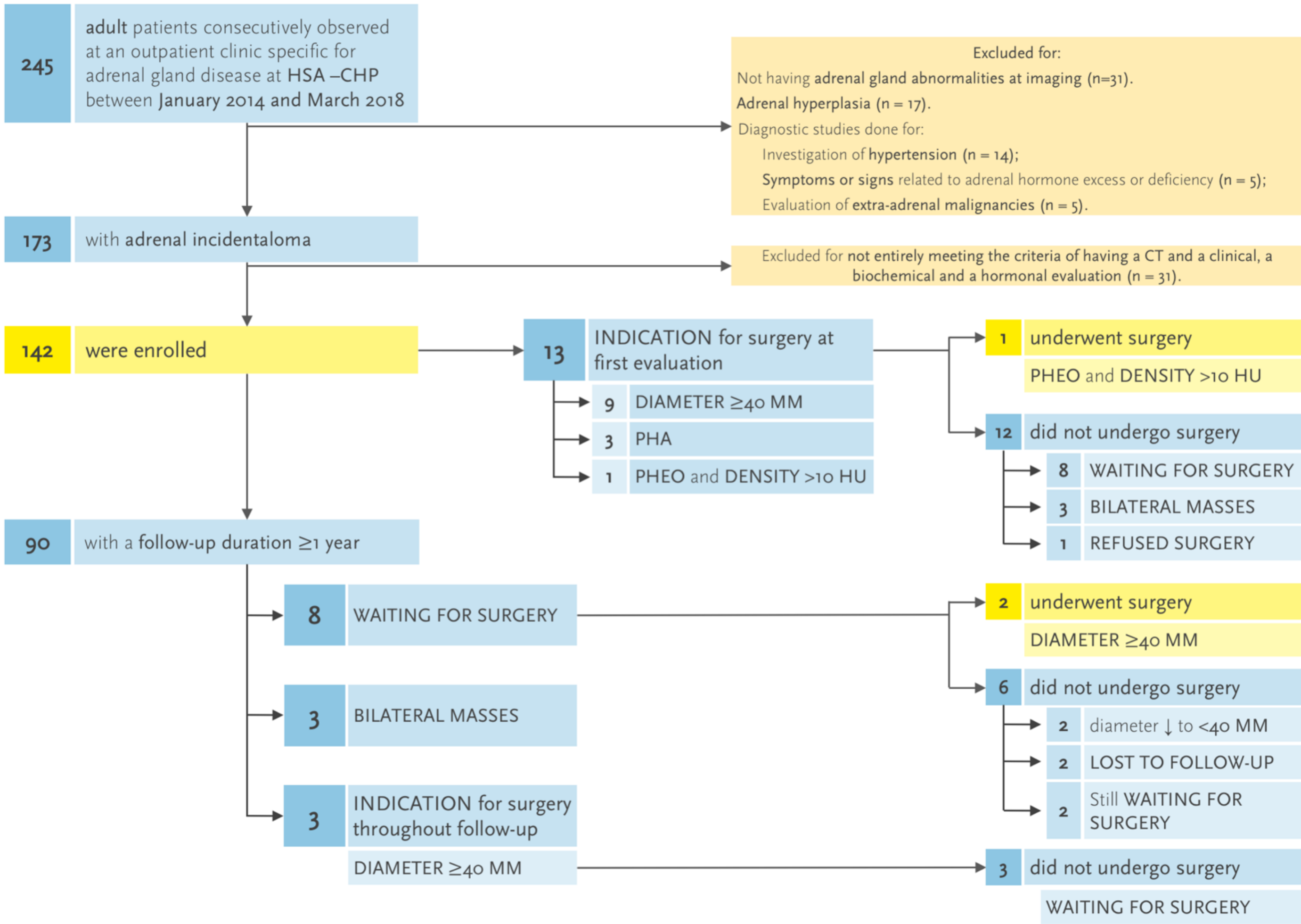
1 mg DST: overnight low-dose dexamethasone suppression test; AI: adrenal incidentaloma; ACS: autonomous cortisol secretion; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PACS: possible autonomous cortisol secretion; SD: standard deviation; *n=65 at diagnosis and 34 five years after; **n=37 at diagnosis and 18 five years after; ***Median (Interquartile range).

**Table VI:** Computed tomography scan features at diagnosis grouped by cortisol production.

| | Non-functioning AI (n=65)* | | PACS or ACS (n=37) | | P-value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| **Mass location** | | | | | 0.976 |
| Left adrenal gland | 29 | 44.6 | 17 | 45.9 | |
| Right adrenal gland | 26 | 40.0 | 14 | 37.8 | |
| Bilateral | 10 | 15.4 | 6 | 16.2 | |
| **Number of masses** | | | | | 0.556 |
| One | 52 | 80.0 | 29 | 78.4 | |
| Two | 11 | 16.9 | 5 | 13.5 | |
| Three | 2 | 3.1 | 2 | 5.4 | |
| Four | 0 | 0 | 1 | 2.7 | |
| **Maximum diameter, mm** | | | | | 0.371 |
| Mean (SD) | 21.0 | (10.3) | 24.1 | (11.4) | 0.145 |
| < 10 | 1 | 1.6 | 1 | 2.7 | |
| 10-19 | 31 | 48.4 | 14 | 37.8 | |
| 20-29 | 23 | 35.9 | 11 | 29.7 | |
| 30-39 | 6 | 9.4 | 7 | 18.9 | |
| ≥ 40 | 3 | 4.7 | 4 | 10.8 | |
| **Non-contrast attenuation coefficient, Hounsfield units**** | | | | | 0.085 |
| ≤ 10 | 47 | 88.7 | 29 | 100 | |
| > 10 | 6 | 11.3 | 0 | 0 | |

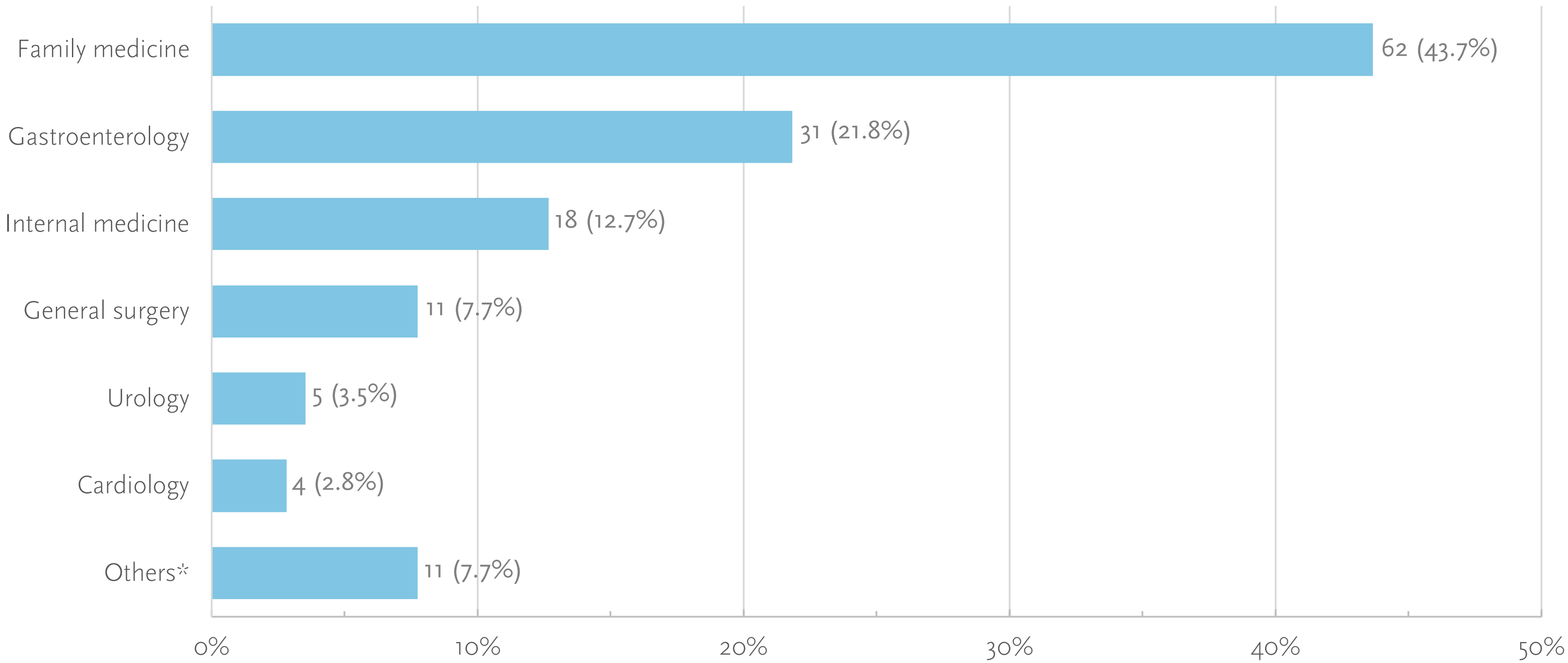
AI: adrenal incidentaloma; ACS: autonomous cortisol secretion; PACS: possible autonomous cortisol secretion; SD: standard deviation; *Data concerning maximum diameter and mass density was not available for 1 patient because we did not have access to the full report or the exam; **For 20 subjects (12 with non-functioning AI and 8 with PACS or ACS) the radiologist did not report non-contrast attenuation coefficient and we could not access to the diagnostic exam.

# FIGURES



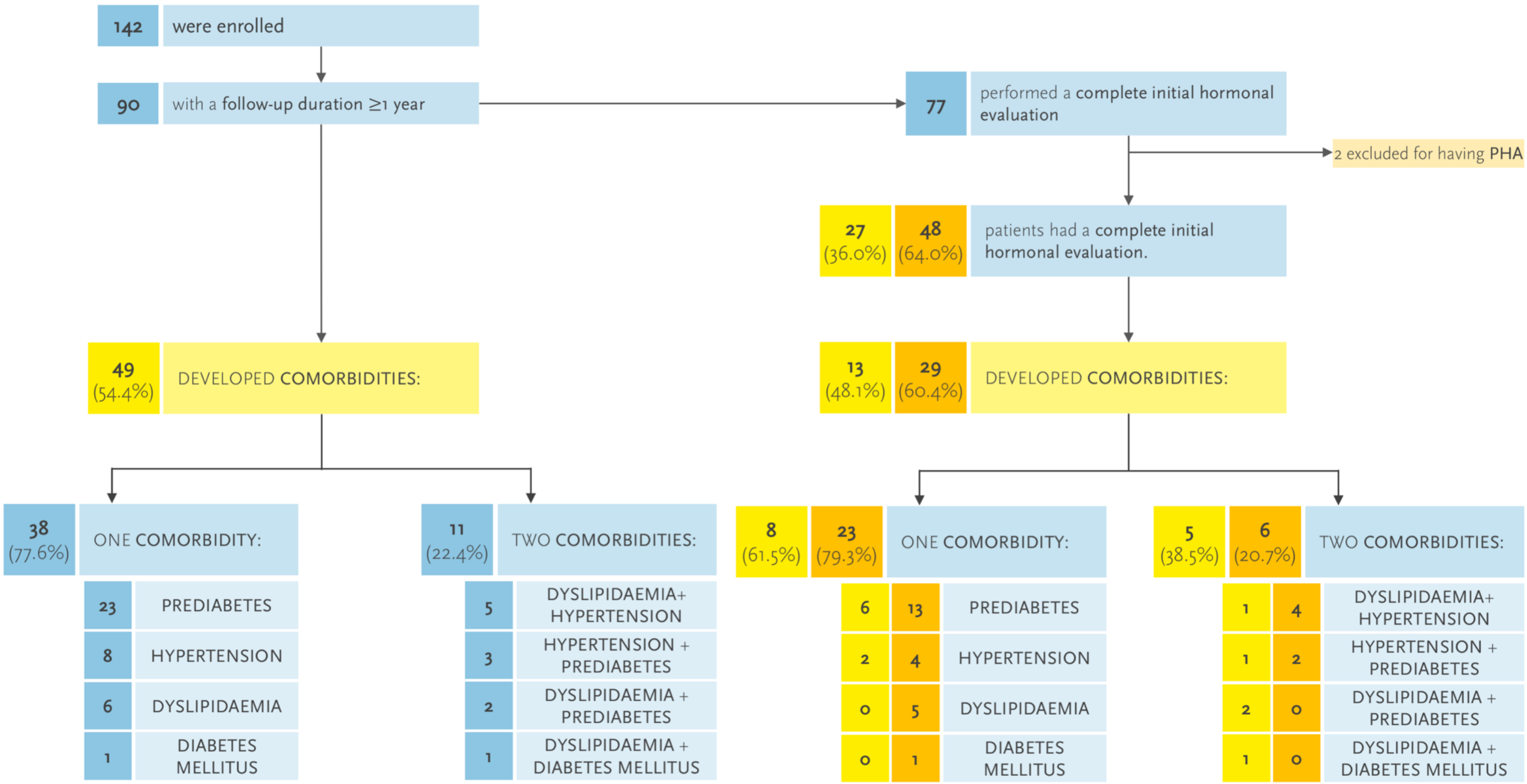

PHA: Primary hyperaldosteronism; PHEO: pheochromocytoma; ↓: decrease.

**Figure 1:** Flow-diagram of patients' inclusion, patients with indication for surgery at diagnosis and during follow-up and patients who underwent surgery.



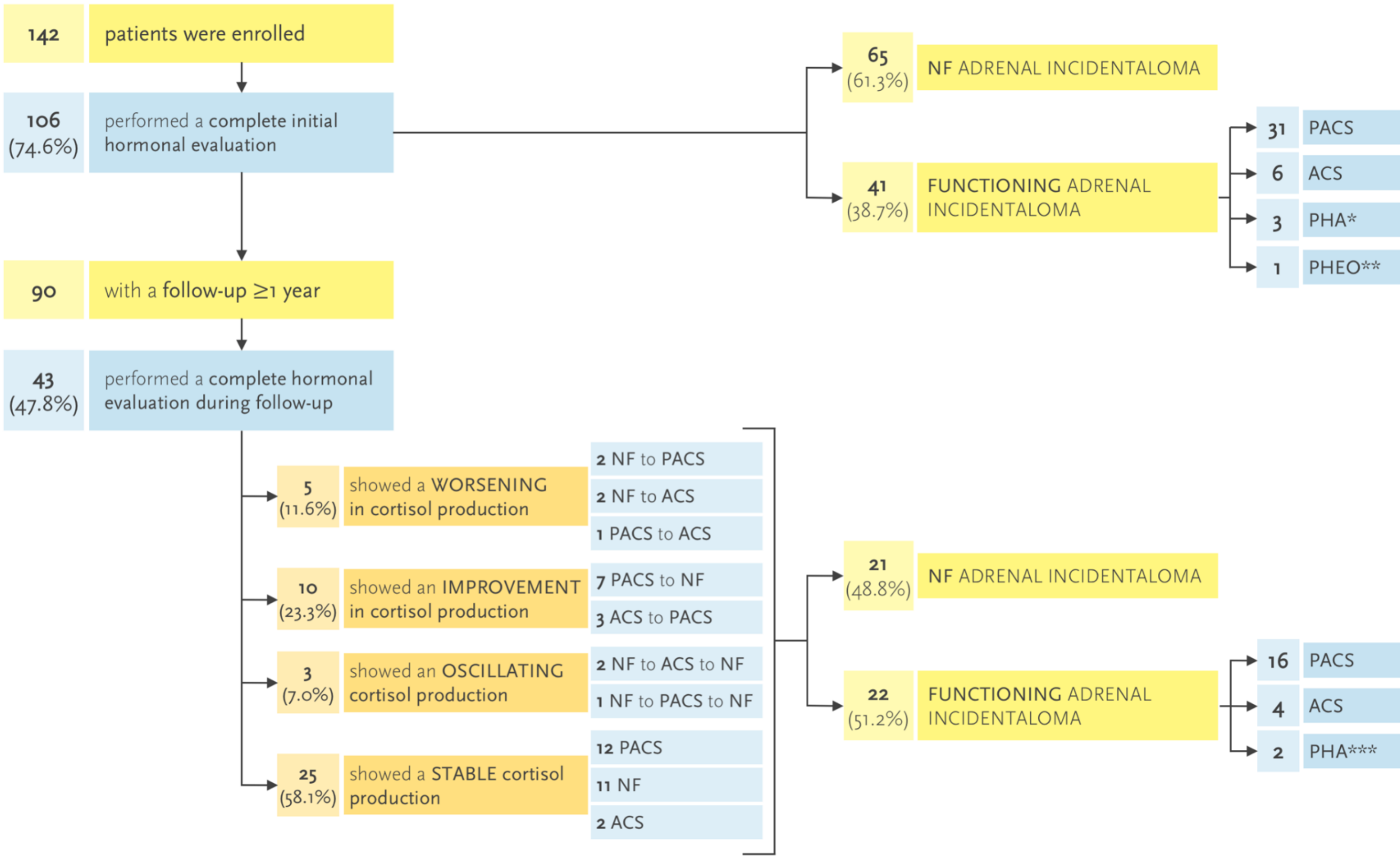

n (%); *Other specializations included infectiology (3 patients), nephrology (3), endocrinology (2), haematology (1), neurology (1) and physical medicine and rehabilitation (1).

**Figure 2:** Specialties of the physicians who referred the patients.




On the right side of the figure, numbers concerning patients with possible autonomous cortisol secretion or autonomous cortisol secretion are inside yellow squares and those concerning patients with non-functioning adrenal incidentalomas are inside orange ones. PHA: Primary hyperaldosteronism.

**Figure 3:** Diagram with patients who developed comorbidities throughout follow-up in global and grouped by cortisol production.




ACS: Autonomous cortisol secretion; PACS: Possible autonomous cortisol secretion; PHA: Primary hyperaldosteronism; PHEO: pheochromocytoma; NF: Non-functioning; *One also had PACS and another ACS; **Also had PACS; ***One also had PACS and the other ACS.

Figure 4: Diagram with the classification of patients according to hormone production at diagnosis and at the end of follow-up and with the change in cortisol production throughout follow-up.